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SHORT COMMUNICATION

Macrotyloma axillare and *M. uniflorum*: descriptor analysis, anthocyanin indexes, and potential uses

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Abstract The purpose of this study was to characterize and identify potential new uses for perennial horsegram (*Macrotyloma axillare*) and horsegram (*M. uniflorum*) germplasm in the USDA, ARS collection. Seven morphological and yield parameters were studied in 11 *M. axillare* and 32 *M. uniflorum* accessions. A wide variation was found in stem branching, foliage, plant height, seed number, and seed weight. Leaf anthocyanin indexes were significantly higher than control flowers from PI 345729. Phytochemical traits exist in both species for potential use as nutraceuticals, forage, and food for humans.

Keywords Characterization · Genetic Resources · Horsegram · *Macrotyloma axillare* · *M. uniflorum* · Perennial horsegram

Introduction

Both *Macrotyloma axillare* (E. Mey.) Verdc. and *M. uniflorum* (Lam.) Verdc. are in the Leguminosae (Fabaceae) family and Phaseoleae tribe. Both *M. axillare* and *M. uniflorum* are self fertile and cleistogamous. The diploid chromosome number for *M. axillare* is $2n = 20$ while *M. uniflorum* has diploid chromosome numbers of $2n = 20$, 22, 24 (Cook et al. 2005).

Macrotyloma axillare is a trailing plant with trifoliolate leaves that are slightly glossy on the upper surface. The raceme consists of white, green to yellow flowers with oblong to elliptical standards. Pods are oblong, laterally flattened, and contain 5–9 subovoid, smooth, buff, red to brown with sparse to dense black mottled seeds capable of producing 50,000–200,000 seeds per kg. *Macrotyloma uniflorum* has longer stipules than *M. axillare*. Leaflets are acute or slightly acuminate, tomentose on both surfaces, and usually produces fewer seed (33,000–75,000 seeds per kg) (Cook et al. 2005). *Macrotyloma axillare* is also known as lime yellow pea and *M. uniflorum* has common names of horse gram, madras bean, poor man's pulse, and kulat as well (Cook et al. 2005).

Both *M. axillare* and *M. uniflorum* can be grown in a range of soils, however, *M. axillare* is less tolerant of heavy clays. Both are very drought tolerant but do not tolerate flooding nor frost, and require an average annual temperature ranging from 18 to 27°C. *Macrotyloma uniflorum* is a short day and day neutral plant maturing 120–180 days after planting, however *M. axillare* exhibits juvenile flowering during the first year of growth with optimum flowering and seed production in succeeding years reaching maturity 30–60 days after flowering (Cook et al. 2005).

The objectives of this study were (1) to characterize *M. axillare* and *M. uniflorum*

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accessions for percentage of branches and foliage, plant size, and seed regeneration in Griffin, GA, USA; (2) analyze anthocyanin indexes recorded from *M. uniflorum* accessions in 2006; and (3) to discuss potential nutraceutical, medicinal, and agricultural uses.

Materials and methods

Characterization and regeneration

Seeds from each of 39 accessions of *M. axillare* and *M. uniflorum* were planted in 6.4 cm × 7.0 cm jiffy pots (Hummert International, Earth City, MO) containing Metro Mix 200 potting soil (Scotts Sierra Horticultural Products Company, Marysville, OH) each year (2001–2006) during March through May in a greenhouse at a range of 21–26°C. After 30–90 days, *M. axillare* and *M. uniflorum* plants were transplanted to field regeneration plots at Griffin, GA. Some accessions from both species were trellised to verify optimum seed regeneration. Plant and seed characteristics were recorded each year from all accessions. Twenty-five to 50 plants representing each accession per plot were transplanted in one 6 m row plot with 3–6 m between rows. Plots were irrigated using sprinklers as necessary. Descriptor data including branching, foliage, plant height, and width (cm) were recorded from plants in each plot at 50% flowering. Branching and foliage were based on a scale of 1–5 where, 1 = >90%, 2 = 80–89%, 3 = 70–79%, 4 = 60–69%, and 5 = 50–59% of each plant producing branches and/or foliage based on visual observation. Mature pods were harvested from each accession 3–6 months after transplanting, dried at 21°C, 25% RH for about 1 week, and threshed. Seeds were then counted, weighed, and seed mass determined by dividing the seed total weight by the total number of seed.

Anthocyanin indexes

Each regeneration plot of *M. uniflorum* in 2006 consisted of 11 accessions growing in one 6 m row and three randomly selected plants in a completely randomized design. *Macrotyloma uniflorum* flowers are near white and will be used as controls. An Opti Sciences CCM-200 chlorophyll content meter was converted to an experimental hand held anthocyanin meter. The manufacturer replaced the 655 nm light emitting diode (LED) of the CCM with a 520 nm LED in order to measure absorbance near the wavelength at which free anthocyanin aglycones in beans, cyanidin and pelargonidin monoglucosides absorb (Macz-Pop et al. 2004). Prior to 50% seed maturity, anthocyanin indexes will be recorded from each of three leaves and flowers (PI 345729) from *M. uniflorum* accessions growing in the field on July 26, 2006. Data for anthocyanin indexes will be analyzed using analysis of variance ($P < 0.001$) (SAS Institute, Cary, NC). Mean separations will be conducted using Duncan's multiple range test ($P < 0.05$).

Results and discussion

Characterization and regeneration

Plant regeneration was satisfactory for all accessions tested. Percentage of each plant producing branches and foliage in both *M. axillare* and *M. uniflorum* ranged from 50 to greater than 90%, respectively. Plant height (measured from soil surface to the end of main stem) within rows ranged from 20 to 180 cm for *M. axillare*. The *M. axillare* accessions growing on trellises and producing the tallest plant over all four years were PI 553014 (180 cm), PI 337654 and PI 420860 (130 cm), PI 415809 (120 cm) and PI 355917 (100 cm). Plant widths (measured from end of main stem) ranged from 50 to 150 cm for *M. axillare* and the widest plant sizes were observed in PI 316454 and PI 420860 (150 cm) followed by PI 364785 (115 cm) and PI 316454 (100 cm). The trellised *M. uniflorum* accessions producing the tallest plants were PI 179688 (150 cm), PI 165581 (140 cm), and both PI 165942 and PI 180292 (100 cm). The widest average of 155 cm was observed in PI 163321, PI 180437, PI 196290, PI 267705, PI 365425, and PI 271042. Coefficients of variation for branching, foliage, plant height, and plant width were 55, 37, 74, and 27% for *M. axillare* indicating greater variability for plant height between all accessions. Coefficients of variation for branching, and foliage were 82 and 76% respectively in *M. uniflorum*. Lower coefficients of variation were found for plant height (58%) and width (44%). This indicates that branching and foliage were both highly variable as well between *M. uniflorum* accessions.

Seed numbers and weights per plot ranged from 5 to 2,979 and 0.31 to 33.1 g, respectively, while seed mass ranged from 0.008 to 0.012 g per seed for *M. axillare*. Both *M. axillare* accessions PI 337654 and PI 415809 produced 2,979 and 2,319 seeds, respectively. Seed weights for both accessions reflected high seed numbers because both weighed an average of 29 g per plot. Eleven *M. axillare* accessions produced 5–749 seeds weighing an average of 75 g per plot. Seed numbers produced by *M. uniflorum* accessions ranged from 4 to 8,400 weighing 3.42 to 341.05 g per plot. The highest seed numbers among *M. uniflorum* accessions were produced from PI 196290 (8,400 seeds), PI 165901 (7,366 seeds), and PI 165581 (6,817 seeds). Total seed weights for these three accessions averaged 293.4 g per plot. Twenty-eight *M. uniflorum* accessions produced 4–5,416 seeds weighing an average of 85 g per plot. Seed mass is a good indicator of seed size. For both species, seed mass ranged from 0.008 to 0.050 g per seed with averages of 0.0096 and 0.039 g per seed for *M. axillare* and *M. uniflorum*, respectively.

The coefficients of variation for seed number and weight were 133 and 114% for *M. axillare*. These values indicate greater variability for seed number and weight than for the other parameters in *M. axillare*. Coefficients of variation for seed number and weight were 113 and 95% in *M. uniflorum.* These values indicate that seed number and weight were more variable in *M. uniflorum* accessions than the other traits tested as well.

The percentage of each plant producing branches was significantly correlated with percentage of each plant producing foliage ($r = 0.9229$) and seed weight significantly correlated with seed mass ($r = 0.7050$) in *M. uniflorum* while seed number was highly correlated with seed weight in both *M. axillare* ($r = 0.9981$) and *M. uniflorum* ($r = 0.9719$). Since plant height, width, seed number, seed weight, and seed mass are quantitative characters, the variability between horsegram as well as perennial horsegram accessions is probably due to segregating accessions as well as the environment in which they were regenerated.

Anthocyanin indexes

Anthocyanins are responsible for color pigments of most flowers and fruits. These pigments occur as glycosides (anthocyanins), and their aglycones (anthocyanidins) (Bruneton 1999). Anthocyanin rich foods have shown potential in providing health benefits. Anthocyanin rich extracts from chokeberry inhibited colon cancer (Zhao et al. 2004). Anthocyanins are potent antioxidants (Wang et al. 1999) and shown to be anti-inflammatory antioxidants by acting as free radical scavengers (Polya 2003). Relative estimates of anthocyanin content based on the CCM-200 indexes recorded from leaves of 11 *M. uniflorum* accessions regenerating in the field ranged from 5.2 to 12.9 while the standard control flowers of PI 345729 ranged from 1.1 to 1.3 (Table 1). These leaf anthocyanin indexes recorded in July 2006 contribute to pest deterrence. Leaf anthocyanin indexes were significantly higher than those recorded from control flowers of PI 345729. An average leaf anthocyanin index of 8.9 was observed from all accessions.

Table 1 Anthocyanin indexes for *Macrotyloma uniflorum* accessions regenerated in 2006

		Anthocyanin index[a]		
		Range		
Accession no.	Organ	Minimum	Maximum	Mean
PI 345606	Leaves	8.7	10.1	9.633[b]
PI 345729	Leaves	6.7	12.9	9.200[b]
PI 337656	Leaves	7.5	11.4	8.800[b]
PI 246404	Leaves	7.0	9.5	8.500[b]
PI 180289	Leaves	5.9	10.0	8.100[b]
PI 246403	Leaves	5.5	10.5	7.967[b]
PI 271042	Leaves	7.2	8.2	7.733[b]
PI 212636	Leaves	5.8	8.6	7.500[b]
PI 277736	Leaves	5.2	9.8	7.367[b]
PI 297892	Leaves	6.0	8.6	7.367[b]
PI 330577	Leaves	5.8	8.5	7.267[b]
PI 345729 (Control)	Flowers	1.1	1.3	1.200[c]
Std. error		0.5	0.8	0.6
C.V. (%)		31	31	28

[a] Relative estimates of anthocyanin content based on the CCM-200 indexes recorded from leaves of 12 *M. uniflorum* accessions. Analysis of variance showed significance differences within accession means ($P < 0.05$). Means within a column followed by the same letter indicate no difference, Duncan's multiple range test, $P < 0.05$ ($n = 3$)

Though limited anthocyanin index levels have been found in these *M. uniflorum* accessions, significant levels occur in leaves when compared with the standard control near white flowers. This is the first report for anthocyanin index levels in *M. uniflorum* accessions. Further research is needed to identify specific anthocyanins in *M. uniflorum* as well as anthocyanin variability across environments. However, this data provides scientists with initial anthocyanin indexes within these accessions.

Food and potential nutraceutical uses

Macrotyloma uniflorum is usually grown for livestock and human food as a pulse (Cook et al. 2005). The phytochemical, D-pinitol is found in *M. axillare* seed (ILDIS 1994) and identified to reduce postprandial (after a meal) blood glucose in patients with type 2 diabetes (Kang et al. 2006). Insoluble dietary fiber is required for normal lower intestinal function in humans (Anderson et al. 1994). Seeds of *M. uniflorum* contain more insoluble dietary fiber than kidney bean (*Phaseolus aconitifolius*) (Kawale et al. 2005). Extracts from *M. uniflorum* seeds had significant activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* (Gupta et al. 2005). *Macrotyloma uniflorum* could play a role in antioxidation as well. Reddy et al. (2005) discovered that when *M. uniflorum* plants were exposed to toxic levels of lead, several enzymes showed a pivotal role against oxidative injury. Sheep appeared to prosper best when fed hay containing 70% *M. uniflorum* than hay consisting of 70% *Stylosanthes hamata*, *Vigna unguiculata*, and *Crotalaria juncea* (Murthy and Prasad 2005).

Conclusion

This is the first report for regeneration, anthocyanin indexes, and potential uses of both *M. axillare* and *M. uniflorum* accessions. Genetic resources of *M. axillare* and *M. uniflorum* regenerate well in the Griffin, Georgia area. It has been determined that *M. axillare* and *M. uniflorum* germplasm from this group vary in seed number, seed weight, branching, foliage, and plant height. *Macrotyloma uniflorum* has the greatest potential for further utilization as food for malnourished and drought-prone areas of the world. In addition, *M. uniflorum* can be used for forage and nutraceuticals because their leaves contain additional health enhancing traits such as anthocyanins. Further research for discovery of additional health enhancing traits from *Macrotyloma* species using high performance liquid chromatography is needed.

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